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Disease interaction and pathogens exchange between wild and farmed fish populations with special reference to Norway

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Disease interaction and pathogens exchange between wild and farmed fish populations with special reference to Norway.

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Abstract

Norway has the largest salmon-farming industry in the world, an industry that is still growing, and in recent years production of marine species like Atlantic cod has also increased. At the same time, Norway has the world's largest wild stock of Atlantic salmon and has fjord systems and ocean areas rich in wild marine fish species which form the basis of a large fishing industry. As the aquaculture industry grows and diversifies, there is concern about the potential effects of pathogens spreading from farmed fish to wild populations. The overall health situation in Norwegian aquaculture is good, but some pathogens are not controlled effectively. In particular, salmon lice produced in farms may cause problems for wild salmonids and other parasites may be abundant too. Also, viral diseases in Atlantic salmon and bacterial diseases in Atlantic cod give rise to several disease outbreaks annually. The open design of most aquaculture systems allows the transmission of pathogens from the environment or from wild fish to the farmed fish. The objective of this review is to provide an overview of current knowledge of disease interaction and pathogen exchange between farmed and wild fish populations, with emphasis on Norwegian condition. In addition, the paper contains an evaluation of research methods that would be useful in expanding knowledge of

pathogen exchange between wild and farmed fish, and in surveys of diseases in wild fish populations. The impact of pathogen transfer from farmed fish to economically important wild fish populations is assessed together with risk analysis considering possible consequences of pathogen exchange between farmed and wild fish. Finally, the review contains suggestions for future research that will increase the knowledge in the field.

Key words: Disease interaction; pathogen exchange: farmed fish; wild fish

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1. Introduction

The fisheries industry has traditionally been very important industry in Norway and in the last 30 years the salmon aquaculture industry has developed tremendously. The export value of farmed seafood now exceeds that of wild caught seafood such that in 2009 Norway exported farmed seafood valued at NOK 25.9 billion, compared with wild caught seafood exports of NOK 18.7 billion (Norwegian seafood export council/www.seafood.no).

Efforts are continuously being made to reduce the risks of pathogen transmission from aquaculture sites and there is great concern about the potential effects of diseases spreading to wild populations. Likewise, there is a risk of diseases spreading from wild to farmed fish, with subsequent proliferation and spread of pathogens in the farms as well as into the environment. However, current debates are not always funded on facts and there is a need in both the fisheries and aquaculture industries for an overview of knowledge in this field. Some recent reviews and reports covering part of the subject – for example ”Salmon Aquaculture Dialogue Working Group Report on Sea lice” (Revie et al., 2009), ”Salmon Aquaculture Dialogue Working Group Report on Salmon Disease” (Hammell et al., 2009) and ”Review of fish disease interactions and pathogen exchange between farmed and wild finfish and shellfish in Europe” (Raynard et al., 2007).

The objective of this review is to provide an overview of current knowledge of disease interaction and pathogen exchange between farmed and wild fish populations, with emphasis on Norwegian conditions. As well as a review of pathogens there is an evaluation of research methods that can be used to increase knowledge of pathogen exchange between wild and farmed fish, diseases in wild fish populations and the impact on economically important wild fish populations. A risk analysis considering possible consequences of pathogen exchange between farmed and wild fish is also included with suggestions for future research.

2. Pathogen review

Introduction - the health situation in farmed and wild fish in Norway at present

The National Veterinary Institute publishes an annual report, the Farmed Fish Health Report, concerning the health situation in Norwegian aquaculture and this also contains some information about wild fish populations. In 2009, the largest disease-related losses in the seawater phase of salmonid culture were virus-associated. The highest number of outbreaks were due to infectious pancreatic necrosis (IPN), pancreas disease (PD) and heart and skeletal muscle inflammation (HSMI) with both PD and HMSI apparently spreading to new areas (Bornø et al., 2010). Cardiomyopathy syndrome (CMS) was also involved in several outbreaks. The causative agents of HMSI and CMS are confirmed to be viruses very recently (Lovoll et al., 2010; Palacios et al. 2010), but at the moment information about these agents are scarce and they will therefore not be included in this review. Gill disease and other diseases of mainly unknown aetiology continue to cause some mortality in farmed salmonids, but these will not be described either. Bacterial diseases do not represent a major problem in farmed salmonids compared to viral diseases, but they constitute the main disease problem in marine farmed fish in quantitative and economic terms. Disease outbreaks due to atypical *Aeromonas salmonicida* are registered primarily in Atlantic cod (*Gadhus morhua* L.) and halibut (*Hippoglossus hippoglossus). Vibrio anguillarum* and *Francisella noatunensis* cause problems to cod, while a few disease outbreaks are caused by *Moritella viscosa* and *Yersinia ruckeri* in Atlantic salmon (*Salmo salar* L.) and *Flavobacterium psychrophilum* in rainbow trout (*Onchorynchus mykiss*) are recorded (Bornø et al., 2010).

"The Norwegian National Action Plan Against Salmon Lice on Salmonids" was implemented in 1997 as a consensus tool to reduce the impact of sea lice from farmed fish. Important measures in the plan were legal limits for the maximum mean number of lice per farmed fish, compulsory reporting of lice numbers to the authorities, strategic regional treatments against lice and monitoring of salmon lice infections in wild salmonids. In spring 2009 sea lice counts

showed that the average number per fish was the same as in 2002. However as the amount of farmed salmon in the sea had increased since 2002 the total amount of sea lice was also much higher. Coordinated delousing has been performed during the winter season in certain zones of western Norway following directions from the Norwegian Food Safety Authority. Development of resistance in salmon lice against certain anti-parasitic drugs is a cause for concern and a national programme for surveillance of resistance is presently being designed by the National Veterinary Institute (Asplin et al., 2010). Other parasites only cause minor problems, however, the number of salmon farms affected by Parvicapsulosis, caused by *Parvicapsuola pseudobranchiola*, has increased (Bornø et al., 2010; Johansen et al., 2009b).

Little is known about the current status of disease outbreaks in wild fish populations, but furunculosis in wild Atlantic salmon has been recorded regularly in the rivers Namsen, Sandøla and Ferga, all surrounding the Namsen fjord (Johansen et al., 2009b). However, no reduction in the annual catches of wild salmon occurred in these areas (http://www.fishnamsen.no/). Also, anecdotal information suggests that vibriosis is common in several marine species, in particular saithe (*Pollachius virens*) (Egidius 1987), and this predates the establishment of aquaculture in Norway. A national surveillance programme for *Gyrodactylus salaris* investigates wild salmonids from 1-3 locations in each of 100 rivers annually and farmed salmonids from 85 farms bi-annually. The parasite was not detected in any new areas in 2009. Health control of wild-caught salmon for brood stock is also carried out annually. Out of 8 Atlantic salmon positive for IPNV in 2008, 7 were escaped farmed fish (based on scale analysis tests). In 2009 IPNV was isolated from two wild Atlantic salmon. The fish were also tested for *A. salmonicida* and *Renibacterium salmoninarum*, and neither were detected in 2009 (Bornø et al., 2010; Johansen et al., 2009b).

2.1 Viral pathogens

2.1.1 Infectious pancreatic necrosis virus - IPNV

Infectious pancreatic necrosis virus **(**IPNV) is the causative agent of infectious pancreatic necrosis (IPN), a viral disease occurring in all major salmon farming countries. The most affected species to die from clinical IPN are salmonids, but IPN may also occur in farmed non-salmonids such as turbot (*Scophthalmus maximus*) and halibut (OIE, 2006). Susceptibility to IPN generally decreases with age, except for Atlantic salmon smolts which are often affected also after transfer from freshwater to seawater (Jarp, 1999; Jarp et al., 1995; Smail et al., 2006). IPN has been removed from the list of notifiable diseases in Norway and by OIE. However, the fish health services continue to register the disease and in 2009 223 Norwegian aquaculture sites experienced IPN. Mortalities were variable, but some sites had significant losses. Nearly all Norwegian salmon are intraperitoneally (i.p.) vaccinated against IPN. In addition a number of oral vaccines against IPN are used during the juvenile stages. The effect of vaccination in relation to other preventive measures is commonly debated. Management routines and environmental conditions may also affect the outcome of disease (Johansen et al., 2009b).

IPNV has been isolated from farmed and wild fish, water, sediment, birds and shellfish and can be transmitted horizontally in and between freshwater and marine environments by a variety of these reservoirs and vectors (Raynard et al., 2007 and references therein). However, little is known about release of infectious virus from reservoirs. Vertical transmission has been demonstrated in brook trout (*Salvelinus fontinalis*) (Bootland et al., 1991; Bullock et al., 1976; Wolf et al., 1963) and rainbow trout (Dorson and Torchy, 1985), but not yet conclusively demonstrated in Atlantic salmon. During an epizootic of IPN, virus is shed with faeces, urine and from dead and moribund fish (Billi and Wolf, 1969; Wolf et al., 1968). There is increasing evidence that IPNV may be transferred from farmed to wild fish through contact with discharges and products from IPNV-contaminated farms (Bucke et al., 1979;

Hastein and Lindstad, 1991; McAllister and Bebak, 1997; McVicar et al., 1993; Mortensen, 1993; Sonstegard et al., 1972; Wallace et al., 2008). Clinical signs of the disease and/or epizootics have not been reported in wild salmonids, however mortalities associated with IPNV in wild marine fish have been observed in non-salmonids (Atlantic menhaden, *Brevoortia yrannus* (Latrobe) (Stephens et al., 1980) and southern flounder, *Aralichthys lethostigmata* (McAllister et al., 1984). Whether the virus was the specific cause of the mortalities is not clear.

High proportions of the farmed fish undergoing an IPNV infection develop a lifelong persistent infection. Thus, farmed fish may be the most important reservoir of IPNV in the aquatic environment. Sub-clinical covert IPNV infections have also been detected in a wide range of estuarine and freshwater wild fish species. However, viral shedding has only been demonstrated in farmed fish both clinically and persistently infected (Dorson and Torchy, 1981; Hill, 1982; Jensen et al., 2009; McAllister and Bebak, 1997; Munro et al., 1976).

Experimental studies have estimated the effects of chronic IPNV exposure on early life stages of rainbow trout eggs and larvae (Bebak and McAllister, 2009). In an aquatic environment with low fish densities, mortality due to IPNV infections is not expected to occur when virus concentrations are < 1000 pfu (plaque forming units) per litre. On the other hand, continuous input of as little as 10 pfu per litre could cause IPN in aquaculture facilities with high fish densities. The minimum dose required to induce infection in salmon post-smolts was estimated to be less than 0.1 50 % tissue culture infective dose ($TCID_{50}$) per ml by bath immersion (Urquhart et al., 2009). Taken together the data shows that there is a potential for infective virus to be released from wild fish to infect surrounding fish. However, all studies of IPNV in wild fish in relation to aquaculture previously undertaken in Scotland (Munro et al., 1976; Wallace et al., 2005; Wallace et al., 2008), North America (McAllister and Bebak, 1997) and Norway (Brun, 2003) suggest that wild fish have a limited or negligible role in the spread of IPNV to farmed fish. Also, analysis of the persistence of IPNV in Scottish salmon

farms supports the assumption that wild fish reservoirs are probably not important for infection of marine salmon farms (Murray, 2006). The time scale for turnover of infection/non-infection in farms was the period of the fallowing and restocking production cycle. Transmission of IPNV was mostly due to relocation of infected farmed salmon and the proximity of the site to neighbouring infected farms.

2.1.2 Viral haemorrhagic septicaemia virus – VHSV.

VHS is a viral disease primarily affecting farmed rainbow trout, but also farmed turbot and Japanese flounder (*Paralichthys oliveus)*. Outbreaks can lead to high mortality and VHS is listed as a notifiable list 2 disease by the OIE. VHSV has been isolated from 82 different freshwater and marine species throughout the Northern Hemisphere (OIE, 2009; Skall et al., 2005). Mortality due to VHSV has been observed in wild freshwater fish in the Great Lakes (Ammayappan and Vakharia, 2009; Groocock et al., 2007; Lumsden et al., 2007) and in marine fish species along the Pacific North American coast (Meyers et al., 1999; Skall et al., 2005). However, the role of VHSV as a primary causative factor in some of these mass mortalities has been questioned (Elston and Meyers, 2009). There are no reports of VHSV isolation from mass mortalities of European wild fish stocks. The infection may persist sub-clinically in rainbow trout, and reservoirs of infection are cultured or wild fish that are covert carriers. Virulent virus is shed with urine and ovarian fluids (Skall et al., 2005), and once the virus is established in a farmed stock the disease becomes enzootic because of the latent carrier fish. Phylogenetic studies of the nucleoprotein (N) and glycoprotein (G) encoding genes of VHSV isolates have identified four main genotypes (I to IV) (Einer-Jensen et al., 2004; Snow et al., 1999; Snow et al., 2004). Genotypes I-III have been found in Europe and genotype III was until recently only detected in marine fish. In 2007, VHS was detected in a rainbow trout farm in Storfjord in mid-Norway (Dale et al., 2009). The disease was later diagnosed in two other farms in the vicinity in 2007, in further two farms in 2008 and in one farm in 2009. VHSV isolates from all outbreaks belonged to genotype III. All aquaculture

sites in the fjord system, containing cod, saithe and salmon, were examined for VHSV during 2007 and found negative. The Norwegian isolate is the first detection of genotype III in rainbow trout. Immersion trials have confirmed that this virus isolate gives significant mortality in rainbow trout, while in Atlantic salmon mortality was only observed after challenge by injection (Dale et al., 2009). Due to the genotype and the high sequence similarity with VHSV isolates from northern European waters, a marine source is most likely. Genotyping of VHSV isolates from other clinical outbreaks in farmed turbot and rainbow trout in areas previously considered VHS free in Sweden and Finland indicates transfer of VHSV from wild to farmed fish (reviewed in Raynard et al., 2007; Skall et al., 2005). The most probable route of VHSV infection in farmed Atlantic salmon in North America was also through contact with wild fish (Raynard et al., 2007; Skall et al., 2005).

VHSV has its origin in the marine environment and phylogenetic studies indicate that it may have been present in marine fish species long before fish farming was established. No documentation of transfer from farmed to wild marine fish exists (Raynard et al., 2007). In the recent Norwegian VHS outbreak in rainbow trout, 260 wild fish, 50 % of which were herring, *Clupea harengus*, caught in the vicinity of the infected farms were investigated without detection of VHSV (Johansen et al., 2009b). However, genotype Ib was identified in herring caught in the outer part of the same fjord (Duesund et al., 2010).

RNA viruses tend to display very high mutation rates and thereby a high capability of adaptation. Therefore, the presence of VHSV in the marine environment is considered to represent a threat to sea-farmed susceptible species. For instance, VHSV has been detected in many gadoids (Bricknell et al. 2006), and may constitute a threat to cod farming. The fact that this virus continues to be detected with spread to new sites gives rise to concern. In Norwegian waters however, the prevalence of VHSV was very low and only two out of 8395

fish were positive (Brudeseth and Evensen, 2002). Unfortunately, fish from the coastal zone were not included in this survey.

2.1.3 Salmonid alphavirus (SAV)

Salmonid alphavirus (SAV) is the causative agent of pancreas disease (PD) in sea-farmed Atlantic salmon and rainbow trout, and sleeping disease (SD) in freshwater-farmed rainbow trout in Europe. PD has been a continuous problem in Irish salmon production and is an increasing problem in marine salmonids in Scottish and Norwegian aquaculture. SD has been reported in farmed freshwater rainbow trout in France for many years and recently also in other European countries (McLoughlin and Graham, 2007).

SAVs are classified as atypical alpha viruses (Weston et al., 2002) and further divided into 6 genetic subgroups (Fringuelli et al., 2008). Isolates from Norwegian marine salmonids form the third subgroup, SAV subtype 3 or Norwegian salmonid alphavirus (NSAV) (Hodneland et al., 2005). There is only conclusive evidence for direct horizontal transmission, supported by epidemiological and phylogenetic studies (Fringuelli et al., 2008; Kristoffersen et al., 2009; Rimstad et al., 2010). However, one study has described experimental vertical transmission in rainbow trout (Castric et al., 2005) and SAV has been found in salmon eggs and fry at low prevalence (Bratland and Nylund, 2009). Salmon may also be infected by the virus without showing clinical signs or mortality (Graham et al., 2006). Other alpha viruses have intermediate invertebrate hosts, but such has not been described for SAV. The virus has been detected by conventional and Real Time PCR in salmon lice sampled from Atlantic salmon clinically affected by PD (Petterson et al., 2009a). It is not known whether the virus replicates in the lice and theoretically the virus could have been located on the outside of the lice.

In Norway PD is a list 3 disease and a general increase in number of cases has been registered in recent years. Hustadvika (mid-Norway) has until now acted as a geographical barrier for

"natural" northward spread of the disease, although some isolated outbreaks have occurred in the northern part of Norway. In 2009, PD was diagnosed in 75 farms which was a slight reduction from previous years (Bornø et al., 2010). In endemic areas vaccination of 0+ smolts was performed in the autumn of 2008. Due to limited amounts of vaccine available, some farms were not able to vaccinate the fish (Johansen et al., 2009b).

The information about SAV infection in wild fish is limited. Testing of sera from 215 Atlantic salmon (wild or farmed) returning to the north and west coasts of Ireland and from 42 wild salmonids in Norway failed to detect SAV or virus-neutralizing antibodies (Graham, 2005). However, virus-neutralizing antibodies have been detected in sera from saithe close to farmed salmon undergoing a sub-clinical SAV infection (5 of 29 individuals analysed were seropositive) (Graham et al., 2006). No disease was observed in the saithe, but this suggests that inter-species transmission from farmed to wild fish, or *vice versa*, can occur. To date there is no evidence of transmission of SAV between wild and farmed fish. Only limited empirical data are available and the epidemiology of PD and SD in farmed and wild fish remains to be resolved.

2.1.4 Nodavirus

Betanodavirus (NV) is the causative agent of the disease viral nervous necrosis (VNN), or viral encephalopathy and retinopathy (VER), in more than 30 different marine fish species worldwide (Munday et al., 2002). In Norway VNN is a notifiable list 3 disease and outbreaks have been registered in farmed Atlantic halibut (Grotmol et al., 1997), turbot (Johansen et al., 2004b) and recently in farmed Atlantic cod (Hellberg et al., 2010b; Patel et al., 2007). In the period 2006-2009 there were a total of 13 and 4 outbreaks of VNN registered in Norway in farmed cod and halibut, respectively (Bornø et al., 2010; Johansen et al., 2009b). Along the west coast of Norway the virus has also been detected in wild cold water fish species and 22

% (25/114) of the wild Atlantic cod examined were positive for NV by Real Time PCR analysis (Nylund et al., 2008).

NV is transmitted both horizontally and vertically and small juveniles are generally more susceptible than larger fish (Munday et al., 2002). Survivors of an outbreak may become persistently infected virus carriers (Johansen et al., 2004a) and the virus has been detected in 10 % of Atlantic cod from aquaculture facilities with no suspicion or signs of disease (Nylund et al., 2008). Horizontal transmission has been shown experimentally from asymptomatic sea bream (*Sparus aurata*) carriers to healthy sea bass (*Dicentrarchus labrax*) (Castric et al., 2001). It is likely that this also occurs between cold water fish species, although it has not been shown. The virus is stable under environmental conditions in sea water and it is dispersed via water from aquaculture facilities with nodavirus infected fish (Munday et al., 2002; Nerland et al., 2007). In persistently infected adult fish such as cod and halibut (Johansen et al., 2004a; Nylund et al., 2008) the virus may possibly reside until maturation and spawning and thus be transmitted to offspring via eggs or sperm. The use of virus-free brood fish is regarded as essential in controlling the disease. There is no specific evidence for transmission of NV between wild and farmed fish.

2.1.5 Infectious salmon anaemia virus (ISAV)

Infectious salmon anaemia virus (ISAV) is an aquatic orthomyxovirus causing the disease infectious salmon anaemia (ISA) in farmed Atlantic salmon primarily in sea water. ISA is a notifiable list 2 disease. In 2008, ISA was confirmed at 17 Norwegian salmon farms, a considerable increase from previous years. Many outbreaks occurred in one area of Troms county (Johansen et al., 2009b). The disease has been registered in most other salmon producing countries in the northern hemisphere (Canada, UK, USA, Faeroe Islands and

Ireland). Recently, ISA has also become a serious threat to the salmon farming industry in Chile (Godoy et al., 2008).

Until recently, the most important measure in combating ISA in Norway has been eradication of affected populations. Vaccination was allowed in most parts of Norway in the autumn 2010, but a vaccination trial was initiated in 2008 in the exposed region in Troms county.

There are no reports of wild salmonids showing signs of the disease. However, the ISA virus has been found in wild salmonids without clinical signs in Norway and the UK (Plarre et al., 2005; Raynard et al., 2001). The virus isolated from wild salmonids in Norway was not virulent when injected into Atlantic salmon (Plarre et al., 2005). Of 1141 serum samples from Atlantic salmon caught in three rivers in the US 1,2 % were seropositive for ISAV (Cipriano, 2009). Based on ISAV detection in wild Atlantic salmon and trout (*Salmo trutta),* they are considered the most likely natural hosts (Anonymous, 2007). No difference in susceptibility to ISAV was found between wild or farmed Atlantic salmon (Glover et al., 2006). Experimental infection by injection has demonstrated replication of ISAV in several fish species; brown trout (*Salmo trutta*), sea trout, rainbow trout, Arctic char (*Salvelinus alpinus*), herring and Atlantic cod (Anonymous, 2007; Grove et al., 2007; Nylund et al., 2002). This result implies that the importance of wild marine fish as virus carriers needs to be clarified. Epidemiological investigations in Norway supports horizontal transmission of ISAV through sea water (Jarp and Karlsen, 1997; Lyngstad et al., 2008) and vertical or trans-generational transmission has been suggested (Nylund et al., 2007; Vike et al., 2009).

Based on phylogenetic analysis it is claimed that ISAV has been transferred from wild to farmed fish at least three times since the start of salmon farming in the north Atlantic (Nylund, 2007; Nylund et al., 2003). There is no documented evidence that ISAV has been transferred from farmed to wild fish (Nylund, 2007). In the area of Troms County that has

had annual outbreaks, ISAV was detected in some of the escaped farmed salmon caught in rivers in the area (Johansen et al., 2009b). Thus, there may be a potential for transmission from farmed to wild fish, but it is not known whether this has actually caused any impact on the wild fish populations.

2.2. Bacterial pathogens

2.2.1 *Aeromonas salmonicida* subsp. *salmonicida*

Aeromonas salmonicida subsp. *salmonicida* is the causative agent of typical furunculosis mainly in salmonid fish both in freshwater and seawater. The disease has been registered in other fish species but there is no evidence of frequent disease outbreaks in non-salmonids (reviewed in (Raynard et al., 2007). The disease was imported to Norway with infected rainbow trout from Denmark in 1964 and again with infected salmon smolts from Scotland in 1985 (Bornø and Colquhoun, 2009). Furunculosis spread rapidly along the coast all the way to Troms County and in 1992 74 water systems and 550 aquaculture sites were infected (Johnsen et al., 1993). Direct evidence for transmission from farmed to wild fish is scarce, but epizootological data suggest that such transmission takes place. Prior to the epizootic in Norway in 1991-1993 the disease was absent in Norway apart from one single outbreak several years before. The rapid spread to farms and natural water sources was associated with several factors such as escaped farmed fish and natural movement of wild fish in the sea (Johnsen and Jensen, 1994). The bacterium is easily spread horizontally through water. A study modelling transfer of pathogens between wild and farmed fish concluded that transmission of furunculosis is depending on the density of the hosts; it will increase as the number of susceptible hosts increases (Murray, 2009). The disease is also transmitted through carrier fish surviving disease outbreaks or contaminated eggs (Bornø and Colquhoun, 2009). Furunculosis is still a notifiable list 3 disease in Norway, but outbreaks in aquaculture nowadays are rare. The eradication of the disease is most likely due to the introduction of highly efficacious vaccines in 1993 (Hastein et al., 2005; Markestad and Grave, 1997). Thus,

problems with transmission of disease to wild populations have been reduced to a minimum and today furunculosis is not considered a problem to wild or farmed fish in Norway. However, some incidents in wild populations demonstrate that the bacterium is still present in the wild, at least in some areas. Besides almost annual outbreak in rivers in the Namsen fjord (Johansen et al., 2009b), a trout breeding station located in a river in Meløy County in northern Norway experienced furunculosis in 2004. All fish were culled and the station was closed. In 2006 one single wild caught trout with furunculosis was registered in the same area, but no source of infection has been found (Norwegian Food Health Authority 2006).

2.2.2 Atypical *Aeromonas salmonicida*

Atypical *Aeromonas salmonicida* infections in more than 20 farmed and 30 wild fish species have been reported, especially from the regions in the northern hemisphere, but also from Australia and Chile (Bravo, 2000; Hastein et al., 2005; Wiklund and Dalsgaard, 1998). Furunculosis vaccines also protect salmonids efficiently against atypical furunculosis. In Norway the disease is caused by *A. salmonicida* subsp. *achromogenes* in cod and heterogeneous strains of atypical *A. salmonicida* in halibut (Hellberg et al., 2009). A few outbreaks of atypical *A. salmonicida* have been diagnosed in halibut, wrasse (*Labridiae* sp.), wolffish (*Anarhichas minor*), Atlantic salmon and Arctic char in recent years (Farmed Fish Health Report 2009 and 2010). An interaction between wild and farmed fish cannot be ruled out, even though evidence is scarce. Plasmid profiling of many strains both from wild and farmed fish, especially salmonids, show that there might be an epidemiological link between these strains (Sørum et al., 2000). The bacterium can cause a carrier state both in farmed and wild fish and a study of isolates from different geographical areas over a long time period indicate a persistent nature of the infection (Pedersen et al., 1996). Experiments have shown that pathogen exchange may take place and disease is transmitted between different species

(Mikkelsen et al., 2009). Disease outbreaks seems to be stress related (Bravo, 2000; Cornick et al., 1984; Magnadottir et al., 2002).

2.2.3 *Vibrio anguillarum*

Vibriosis, caused by several serotypes of *Vibrio anguillarum*, is a significant disease problem in cod farming in Norway. The number of sites (around 20) with positive identification has been stable since 2003 (Fish Health Report 2009 and 2010). The most frequent serotypes of *V. anguillarum* isolated from cod are O2b followed by O2a while serotype O1 has only been isolated from salmonids (Hellberg et al., 2010a). In 2008 vibriosis caused by *V. anguillarum* serotype O2b was identified in samples from wild-caught saithe (Hellberg et al., 2009). Since the introduction of vaccines which efficiently protect Atlantic salmon against vibriosis, the disease is not a problem to farmed salmon in Norway. Vibriosis occurs in a number of cultured and wild marine fish species and salmonids in salt-, brackish- and freshwater (Egidius, 1987). The bacterium is found both free living and in fish (West and Lee, 1982) and is easily spread through water (Grisez et al., 1996). Direct transfer of disease between wild and farmed fish is not documented, but it is believed that wild fish and prey organisms may be reservoirs for different serotypes (Hjeltnes and Roberts, 1993). Thus, potentially transfer between wild organisms and farmed fish may occur.

2.2.4 *Flavobacterium psychrophilum*

Flavobacterium psychrophilum is the causative agent of bacterial cold water disease in adult salmonids and rainbow trout fry syndrome in juvenile rainbow trout. The disease is found worldwide, especially in freshwater salmonid farming, and its control mainly relies on antibiotic treatments (Nicolas et al., 2008). It is an important limiting disease in European rainbow trout farming (Brun et al., 2009). In Atlantic salmon detections are mainly related to ulceration and fin rot. In Norway, the most severe outbreaks are found in fry and juveniles of

rainbow trout in freshwater, but also in seawater reared rainbow trout. Mortality levels of 90 % has been reported in the smallest fish (<5 g). All affected fry in 2008 and 2009 came from the same egg producer and indicate transmission from the eggs. In seawater rainbow trout both from unaffected freshwater farms and affected freshwater farms have been infected, indicating horizontal transmission (Johansen et al., 2009b). Rainbow trout are now vaccinated with an autogenous i.p. vaccine before being transferred to sea in areas with systemic *F. psychrophilum* infections in fish (Bornø et al., 2010). The bacterium is easily spread through water and reduced water quality and stress may provoke outbreaks (Brun et al., 2009). *F. psychrophilum* has been found in wild fish used as brood stock, wild salmon close to fish farms, in several wild fish species with no clinical signs of disease and in the water environment of fish farms that are infected (Raynard et al., 2007). There are no reports on the disease in wild fish in Europe. Direct evidence of transfer from wild fish to farmed fish has not been found (Raynard et al., 2007).

2.2.5 *Francisella noatunensis*

The intracellular bacterium *Francisella noatunensis,* causative agent of Francisellosis, is at present considered the most important disease problem in Norwegian cod farming. The disease was diagnosed for the first time in 2004 (Nylund et al., 2006; Olsen et al., 2006). *F. noatunensis* has also been detected in historical samples from wild cod caught in the North Sea (Zerihun et al., 2008), and has thus been present in wild cod before the development of modern cod aquaculture. The number of annual registered outbreaks has varied between 7-14 the last four years, most of them in south Norway (Farmed Fish Health Report 2009 and 2010). Francisellosis is a notifiable list 3 disease in Norway. The disease is chronic and can occur in combination with other infections. Other strains of the genus *Francisella* previously isolated from Atlantic salmon in Chile are *F. noatunensis w*hereas *F. asiatica* has been isolated from tilapia and other warm water fish (Birkbeck et al. 2010; Mikalsen and Colquhoun, 2009). The presence of *F. noatunensis* in wild cod populations along the coast of

Norway has been studied using Real Time PCR for the genes 16S rDNA and fopA. *F. noatunensis* was detected in samples from all counties south of Sogn and Fjordane with 13 % prevalence. The authors speculate that the apparent absence in the northern parts of Norway may be due to colder seawater temperature (Ottem et al., 2008). However, it should be emphasized that temperatures in northern Norwegian waters are often well within the ecological niche of *F. noatunensis*. More likely, high temperatures in southern Norwegian waters cause suboptimal functioning of the cod immune system, resulting in increased susceptibility to diseases.

2.2.6 Other bacterial pathogens

Renibacterium salmoninarum, causative agent of bacterial kidney disease (BKD), emerged in wild Atlantic salmon in Scotland in the 1930s (Mackie et al., 1935). Susceptible species are mostly salmonids, but BKD has also been reported in non-salmonid species (reviewed in Raynard et al., 2007). The disease is widespread in Europe, North– and South America and Asia. In Europe infection is rarely detected in wild fish (Raynard et al., 2007). The infection is persistent both in wild and farmed populations. A recent study in Great Britain showed that wild fish populations, especially those in the vicinity of rainbow trout farms with recent BKD, were *R. salmoninarum* positive while the farmed fish were negative (Chambers et al., 2008). In a surveillance of marine rainbow trout farms in Denmark the bacterium was found in five of the eight farms studied (Pedersen et al., 2008). BKD is a notifiable list 3 disease in Norway and a yearly monitoring programme including all salmon brood stock farms is performed to avoid vertical transmission (National Food Safety Authority 2009). This has reduced the number of cases both in seawater and freshwater dramatically over the last 15 years. In 2009 BKD was detected in two rainbow trout farms and one salmon farm (Bornø et al., 2010). As both horizontal and vertical transmission has been reported, there is a risk for transfer between wild and farmed species. Evidence for transfer has been found only in North American trout species in freshwater (Mitchum and Sherman, 1981; Mitchum et al., 1979).

Cold water vibriosis caused by *Vibrio salmonicida* was initially a problem in farmed Atlantic salmon, but has recently occasionally been reported in cod in Norway (Hellberg et al., 2009). The introduction of vaccines has effectively eliminated the disease (Sommerset et al., 2005). Cold water vibriosis is transmitted horizontally and an epizootological study based on plasmid profiles suggested transmission from farmed Atlantic salmon to wild-caught Atlantic cod and *vice versa*. Both species were being held in neighbouring net-pens in a fish farm north in Norway. *V. salmonicida* has not been registered in wild fish populations except for this incident with cod (Sørum et al., 1990).

Moritella viscosa is considered to be the main causative agent of winter ulcer in marine cultured species, even though other bacteria also may be involved in the development of ulcers. In Norway the bacterium was registered on 36 sites in 2009. Vaccination may have reduced losses due to winter ulcer in Norwegian salmon farming, since fewer outbreaks have been reported in recent years. Antibiotics have not proven effective in controlling the disease (Johansen et al., 2009b). *M. viscosa* has been isolated from wild fish species (Benediktsdóttir et al., 2000; Lunder et al., 2000) and farmed Atlantic salmon, rainbow trout and Atlantic cod (Benediktsdóttir et al., 2000; Colquhoun et al., 2004; Lunder et al., 2000). The disease is transmitted horizontally. No impacts have been reported on wild fish species and there is no evidence of interaction between farmed and wild fish. However, as the bacterium has been isolated from wild fish, a potential for interaction exists (Raynard et al., 2007).

Piscirickettsia salmonis is the causative agent of piscirickettsiosis primarily in farmed salmonids. It is one of the most serious diseases in Chilean aquaculture (Almendras and Fuentealba, 1997). The first outbreak in Atlantic salmon in Norway was registered in 1988 (Olsen et al., 1997). Recently there have been very few outbreaks of the disease and in 2009 only one isolation was done in Norway in connection with a PD outbreak (Johansen et al.,

2009b). Norwegian isolates cause much lower mortalities than those isolated in Chile. Horizontal transmission has been reported and vertical transmission may also be possible (See Raynard *et al.* 2007 and references herein). The bacterium has been isolated from wild fish but disease outbreaks in wild populations have not been reported. Evidence of disease transfer between wild and farmed fish does not exist (Fryer and Hedrick, 2003; Raynard et al., 2007).

2.3 Parasites

Introduction

Evidence for transmission of parasites from wild to farmed fish and *vice versa* is scarce. However, many of the parasites found in wild fish may be a potential threat to mariculture. This review differentiates between parasites with direct or indirect transmission. Parasites with direct transmission do not need an intermediate host and infect either by host to host contact or by short periods of free living stages. This group includes pathogens such as *Gyrodactylus* sp., *Trichodina* sp., *Ichthyobodo necator*, and several parasitic arthropod species. Parasites with a direct transmission or transmission patterns that do not require ingestion of an intermediate host may be major threats to mariculture, especially due to high host densities. Parasites with indirect transmission use one or more intermediate hosts to reach their final host where they mature and reproduce. The gastrointestinal parasites of fish are in most cases dependent on the final host ingesting the intermediate host. Dependent on the type of parasite and the stage of the life cycle these intermediate hosts can range from small pelagic copepods via benthic crustaceans to fish of prey. When fish are fed processed food under farming conditions, transmissions of many of the indirectly transmitted parasites is blocked. Parasites with indirect lifecycles have a significantly lower prevalence in aquaculture, but there are still a few infected individuals in the farmed populations (Heuch et al., 2007). There are, however, some notable exceptions to the lower prevalence of indirectly transmitted parasites in farmed fish. Tapeworms (*Eubothrium* spp.) have been known to cause infections in farmed fish through ingestion of the intermediate host (Raynard et al., 2007), and

this has sub-lethal effects on the fish (Bristow and Berland, 1991). In addition, there are anecdotal reports on accidental infections of gastrointestinal helminthes with indirect lifecycles (MacKenzie et al., 2009). Furthermore, the parasitic crustacean *Laernocera branchialis* may be a future pathogen in cod farming and a threat to wild fish. This is an ectoparasite and does not rely on ingestion of an intermediate host, but still has intermediate (flatfish) and final hosts (gadoids) (MacKenzie and Hemmingsen, 2003).

2.3.1 *Ichthyobodo* spp.

The euglenozoan flagellate *Ichthyobodo necator* is reported to infect fish worldwide and causes massive losses to aquaculture. Evidence suggests that *Ichthyobodo* is a species complex with several species preferring different hosts (Callahan et al., 2005). *Ichthyobodo* spp. are reported from salmonids, cod and halibut (Isaksen et al., 2007; Karlsbakk et al., 2009; Rintamaki Kinnunen and Valtonen, 1997) and pose a major threat to both wild and farmed fish populations. The parasite is found on the skin of juvenile salmon in freshwater, and if left untreated can lead to mass mortalities (Poppe, 1999; Rintamaki Kinnunen and Valtonen, 1997). Salmon in sea cages usually show infection of *I. necator* on the gills where the parasite is attached to the secondary lamellae (Poppe, 1999).

2.3.2 *Trichodina* spp.

Trichodina spp. are ciliates found on several fish species. If the intensity on a host increases the host sheds epithelium and the trichodinids start feeding on mucus of the host and thus become parasitic (Poppe, 1999). Several species are known from cod. *T. cooperi* and *T. murmanica* have been reported from cage reared cod in Iceland. Kristmundsson et al. (2006) and Khan (2004) also reported mortalities in cod caused by *T. murmanica*. In addition, *T. hippoglossi* can infect farmed halibut, reaching up to 1000 individuals per halibut larvae (Nilsen, 1995).

2.3.3 *Spironucleus* spp.

Both farmed salmonids and gadoids can be infected with the diplomonad flagellate S*pironucleus* sp. (Poynton et al., 2004). Infections occur within the gut and to a lesser extent in the skin. The parasite is not common in farmed fish in Norway, but is abundant in wild cod (Heuch et al., 2007). In salmon farms, infection can lead to disease and morbidity (Poppe et al., 1992). *S. torosa* is common in cod, but does not seem to cause any harm (Poppe, 1999).

2.3.4. Myxozoa

Myxozoans are metazoan, extracellular parasites of fish and other vertebrates (Bush et al., 2001; Karlsbakk et al., 2002). *Parvicapsula pseudobranciola* infects the pseudobranch of salmon and has been reported to cause mortality in farmed fish (Karlsbakk et al., 2002; Nylund et al., 2005). Several species of Myxosporidian parasites are reported from gadoids, Atlantic cod, whiting (*Merlangius merlangus* (L.), and haddock, (*Melanogrammus aeglefinus* (L.) (MacKenzie et al., 2005). It is assumed that most myxozoan parasites have an aquatic invertebrate intermediate host, but not all life cycles are known (Bartholomew et al., 2006). MacKenzie et al (2005) investigated gadoids in the north Atlantic and found nine species of myxosporean parasites belonging to the genera *Ceratomyxa*, *Leptotheca*, *Myxidium* and *Sphaeromyxa*. The lifecycles of these parasites are mostly unknown, but often a polychaete or oligochaete act as an intermediate host (Køie et al., 2004). Myxosporean parasites in gallbladders of cod are listed as one of the disease problems that can occur in gadoid farming (MacKenzie and Hemmingsen, 2003; MacKenzie et al., 2005).

2.3.5 Microsporidia

The microsporidia are unicellular parasites that infect most animal groups, and all classes of vertebrates. This group of parasites are obligate intracellular parasites which infect host cells through a polar tube (Bush et al., 2001; Canning and Lom, 1986). They have traditionally been classified as primitive eukaryotes, however recently these parasites were classified as

fungi (Keeling and Fast, 2002 and references therein). The microsporidian parasite *Loma brachialis* is relatively pathogenic at high intensities and has been reported to cause mortalities and reduced growth in cod in Newfoundland (Khan, 2005). The parasite is present in Norwegian aquaculture but has not yet attributed to any losses (Karlsbakk et al., 2009).

2.3.6 Plathyhelminthes

Among the different kinds of flatworms there are three parasitic groups that are interesting to mariculture, 1) the tapeworms which require an intermediate host for dispersal, 2) the digenians which all uses a mollusc as first intermediate host, and 3) the monogenean parasites. The tapeworms (class Cestoidea) represent a large group of intestinal parasites which are found in most fish as well as other vertebrates, including man (Bush et al., 2001; Marcogliese, 1995). These hermaphroditic parasites always rely on an intermediate host to infect their final host (Olson, 2008). Four orders of tapeworms infect fish through ingestion of zooplankton, namely; Trypanorhyncha, Tetraphyllidea, Pseudophyllidea and Proteocephalidea (Marcogliese, 1995). Within the order Tetraphyllidea, *Eubothrium* spp. has been known to cause high infections in both farmed and wild fish populations and has sub-lethal effects on farmed salmonids (Bristow and Berland, 1991).

In mariculture the only reported problems with digenean parasites (Class Trematoda, Subclass Digenea) are those connected to the species *Cryptocotyle lingua* which causes “black spot disease”. These digeneans use snails of the genus *Littorina* as first intermediate host, fish as second intermediate host and seabirds (*Larus* spp.) as final host (Bricknell et al., 2006; Möller and Anders, 1986). *C. lingua* has an Arctic-Boreal distribution among coastal marine fish (Hemmingsen and MacKenzie, 2001) but prevalence varies greatly among different coastal areas from 1.6 % in cod (Buchmann, 1986) to as high as 91.1 % in Swedish sticklebacks (*Gasterosteus aculeatus*) (Barber, 2003). Infections are often benign, but reduced quality in fish fillets can pose a problem if fish are heavily infected (80-400 individuals) (Buchmann,

1986). Larval fish and juveniles might be seriously affected if the brain or the heart is infected (Möller and Anders, 1986). Elevated levels of infection have been found in wild fish, as well as snails around fish farms near shore (Dempster et al., 2009; Kristoffersen, 1991).

Mongeneans (Class Monogenea) are, with a few exceptions, ectoparasites on fish and have a direct life cycle. Most monogenean parasites are egg laying and slow swimming onchomiracidium larva infect fish. Among the monogenean parasites is one of the most serious fish pathogens in Norway, *Gyrodactylus salaris.* From the genus *Gyrodactylus* over 400 species are described, but the vast majority of species are unknown in Norway (Bakke et al., 2007). Species from this genus are not egg-laying, but have an effective reproduction through parthogenesis. Gyrodactylids are hyperviviparous as they give birth to already pregnant daughter individuals, and thus have the ability to rapidly reach high number of individuals once a host is infected (Bakke et al., 2007). Molecular markers have been used together with morphology to compare closely related species and strains of *G. salaris* (Hansen et al., 2007; Matejusova et al., 2001; Ziętara and Lumme, 2003; Ziętara et al., 2000). Gyrodactylosis has since 1975 been a huge problem to the Atlantic salmon stocks in Norwegian rivers as well as in freshwater rearing of salmon parr (Alonso et al., 2004; Bakke et al., 2007).

Besides *G. salaris*, marine species within the genus poses a threat to aquaculture. From Atlantic cod seven species of *Gyrodactylus* are recorded (Hemmingsen and MacKenzie, 2001). The marine gill parasite *G. marinus* is described from Atlantic cod, Alaskan pollock (*Theragra chalcogramma*) and Pacific cod (*G. macrocephallus)* (Bychowsky and Poljansky, 1953; Malmberg, 1970). There are few reports on the pathogenicity of this species, but farmed cod have higher mortalities when exposed to additional stress (Svendsen, 1991). The oviparous monogenean parasite *Entobdella hippoglossi*, which infects Atlantic halibut can also cause problems in aquaculture (Svendsen, 1991).

2.3.7 Arthropoda

Among animal phyla the Arthropoda is the phylum with highest diversity and species richness. Several arthropods are parasitic on a wide range of hosts. In addition arthropods are vectors for diseases caused by parasites (Bush et al., 2001). One important arthropod, the salmon louse (*Lepeophtheirus salmonis*) causes problems in marine salmon farming in Norway, as well as resulting in severe problems to wild fish stocks (Pike and Wadsworth, 2000). The life cycle of the sea lice generally comprises five phases and 10 stages (Johnson and Albright, 1991; Pike and Wadsworth, 2000; Schram, 1993). Salmon lice have created severe problems for the aquaculture-wild salmon interaction, and this experience may serve as an example of future problems to come. After mating, the long-lived female salmon lice often move to post-anal areas of the host and may extrude up to 11 pairs of sacs with fertilised eggs (Pike and Wadsworth, 2000) for several months. Because each egg sac may contain 100-1000 eggs (see Nordhagen et al., 2000 and Pike and Wadsworth, 2000 for details), a large number of planktonic offspring may be produced over the female's lifespan. This has implications for the infestation dynamics within farms and between farmed and wild host fish.

A few salmon lice epidemics in wild salmonids have been reported before establishment of aquaculture or in areas without fish farms (Johnson et al., 1996; White, 1940). Salmon lice have typically been found at rather high prevalence but low intensity, probably in a quite regulated and stable host-parasite system, and few adverse effects on the host population have been observed (Bjørn et al., 2001; Boxshall, 1974; Krkošek et al., 2005; Krkošek et al., 2006b; Pemberton, 1976; Tingley et al., 1997). However, salmon farming has fundamentally changed the number of salmon lice hosts and also the epidemiology of the host-parasite system (Heuch and Mo, 2001; Heuch et al., 2005). In Norway, the number of salmon lice hosts have increased more than hundred times since salmon farming started (Heuch et al., 2005), permitting adult female lice from farmed fish continuously to produce lice infestation

stages into the surrounding waters (Costello, 2006). Severely increased infestation intensities have been observed in intensively farmed areas (Bjørn et al., 2001; Bjørn et al., 2007; Krkošek et al., 2005; Krkošek et al., 2006a; Krkošek et al., 2006b). In combination with the relatively high clinical impact of salmon lice (Heuch et al., 2005), at least at high intensities and at mobile stages, parasite induced mortality can therefore be expected. In Norway, direct parasite induced mortality in wild Atlantic salmon post-smolts, have been predicted to vary between 0 up to 95 % between years and fjords in the most intensively farmed area of western Norway (Bjørn et al., 2009; Holst et al., 2003). Similar mortality estimates have been predicted for sea trout in intensively farmed areas in Northern Norway (Bjørn *et al.* 2001), as well as for pink salmon (*Oncorhynchus gorbuscha)* in intensively farmed areas of western Canada (Krkošek et al., 2007a; Krkošek et al., 2007b). Even though direct evidence of transmission from farmed to wild fish is hard to find, it is likely that salmon farming causes lice epizootics in Norway. Thus, large numbers of sea lice in fish farms pose a hazard to wild fish and control measures to reduce sea lice numbers in aquaculture are necessary. In addition, several other *Lepeophtheirus* spp., e.g. *L. hippoglossi* on halibut and *L. thompsoni* on turbot (Poppe, 1999), may have the potential to cause future problems in mariculture.

In their review on sea lice and salmonids Pike and Wadsworth (2000) report that approximately 200 species of *Caligus* sp. and *C. elongatus* alone are found in 80 fish species, both elasmobranch and teleost. Several *Caligus* species are thus interesting to aquaculture. Four *Caligus* species are reported from Atlantic cod and infections have also been found in farmed salmonids. Interestingly there are few reports on epizootics on gadoids in aquaculture (Bricknell et al., 2006), but the potential for epizootics is present if gadoid farming grows.

3. Evaluation of research methods

Introduction

Parasitic organisms naturally exist in an unstable equilibrium with their hosts. This equilibrium is affected by environmental changes and anthropogenic activities (c.f. Reno, 1998), such as the development of aquaculture. Generally, knowledge of diseases in wild fish is anecdotal and has arisen from atypical events, such as epizootics where large numbers of fish were affected, or when observations were made on a limited number of fish with no knowledge of the geographical distribution of the condition. Limited research has been undertaken on diseases of wild fish at the population level, and on the interactions between wild and farmed populations in terms of exchange of pathogens, with a few exceptions, for instance in the case of sea lice. Partly, this can be attributed to methodological difficulties associated with the study of diseases in wild fish populations, as infected fish often die and disappear quickly. Despite the general low level of public and scientific interest in fish health and diseases prior to the advance of modern aquaculture, some information was generated early, see for instance the reviews by Egidius (1987) on vibriosis, and the review by Hiney and Olivier (1999) on furunculosis, and references cited therein. In contrast, diseases of cultured fish are well monitored. In Norway, important diseases are listed as notifiable and subject to mandatory reporting including treatments (Lillehaug et al., 2003; Sommerset et al., 2005).

3.1 Methods for detection of pathogens.

Methods should be evaluated and optimized for the specific pathogen to be detected. In case of surveys on pathogens in wild fish populations, it is necessary to apply methods with high sensitivity to be able to detect low levels of pathogens. Sample handling is also of vital importance. Today, methods for detection of pathogens are dominated by very sensitive PCR-based methods, in particular Real Time PCR. The presence of nucleic acids from bacteria, viruses and parasites may be detected using PCR assay with primers specific for genes

common in all strains of a specific species. These methods are also recommended by the OIE for diagnostic purposes (OIE, 2009). However, extensive optimization is required to verify sensitivity and inter-laboratory reproducibility (Julin et al., 2009; Kerr and Cunningham, 2006; Storset et al., 2006). Real Time PCR analyses for detection of viral and bacterial fish pathogens are offered by commercial companies in Norway and this has enabled the aquaculture industry to increase internal controls of pathogen proliferation and transfer between farms. One commercial laboratory also offers Real Time PCR based detection of the parasites *Paranucleospora theridon* and *P. pseudobranchiola.* The companies claim that their procedures are very sensitive, but they do not give the detection limit of each analysis. As a part of the accreditation protocol however, the protocols are available in the public domain. The same policy is practiced by the National Veterinary Institute, which also serves as the national reference laboratory for notifiable diseases according to legislation of the European Economic Area. Surveys of wild fish have, with few exceptions, only been carried out by research institutions.

PCR assays are sensitive methods, but they cannot distinguish between nucleic acids from viable and non-viable pathogens, from vaccine components and free residual bacterial/viral nucleic acids, or (normally) from virulent or non-virulent pathogens. Isolation of viable viruses, bacteria and parasites from fish is also commonly applied. Cell cultures used for virus isolation vary greatly in their susceptibility to different viruses. The amount of infectious virus in the sample and the sensitivity of the cell line are major factors affecting the outcome of virus isolation. Bacteria can usually be cultured in specific growth media/agar plates. For diagnostic purposes pathogen isolation is often used in combination with antibody based methods and PCR for specific detection. Compared to detection of viral or bacterial nucleic acids by PCR based methodology, virus/bacterial isolation detects infectious viral particles and live bacterial cells and also enables quantification of these by titration. On the other hand, PCR methods may detect viral/bacterial nucleic acids even though infectivity in cell culture or

viability may have been lost or reduced due to suboptimal sampling and environmental factors, or if the pathogen isolate is a low virulence variant unable to propagate outside the host. However, the significance of positive findings of viruses or bacteria by PCR alone for the risk of developing the disease and for the risk of shedding and transmitting infectious pathogens is not clear. It is also important to consider which organs are suitable for pathogen detection. This requires basic knowledge of the tissue tropism of each specific pathogen and may also vary due to whether samples are from diseased or asymptomatic carrier fish (Andersen et al., 2007; Korsnes et al., 2009).

Non-lethal methods would be very useful and desirable for the detection of pathogens in wild fish. Bowers et al. (2008) used Real Time PCR and found that IPNV load in pectoral fin tissue was comparable to the viral load in spleen and head kidney tissue, indicating that pectoral fin could be used for the detection and quantification of IPNV. To decide whether pectoral fins could be used in wild fish surveys, they should be tested in cell cultures for the presence of infective viruses. Serological methods have been used in some field surveys. These methods measure antibody responses in serum samples and thus require only non-lethal sampling. For instance, Knüsel et al. compared the detection of VHSV and IHNV by virus isolation and Real Time PCR in samples from a field survey and found that Real Time PCR was applicable for field surveys and slightly more sensitive than virus isolation. However, a serum neutralization test detected much higher numbers of positive cases (Knüsel et al., 2007). Antibody levels can be measured months after contact with the antigen, but it is uncertain which precautions should be taken when fish have elevated antibody titres and test negative for the presence of viruses.

The cornerstone of parasitology is a correct identification of parasites within a host population. Methods that are commonly used to detect and classify parasites are visual detection, light microscopy, scanning or transmission-electron microscopy or genetic

methods. Alpha taxonomy is of major importance both for academic purposes and to mariculture in general. Advances in molecular biology have greatly increased knowledge about taxonomic relationships as well as giving effective tools for identification of pathogens. The use of molecular markers has been applied for identification of parasitic organisms with complex or even cryptic morphology (Hansen et al., 2007).

3.2 Methods for epidemiological studies of pathogens.

To understand the diversity and dissemination of infectious agents molecular methods have become a very important part of epidemiological studies. Sequence data are used to deduce phylogenetic relationships between pathogens. These data may also be used to monitor eradication of well-known pathogens and emergence of previously unknown pathogens (Hungnes et al., 2000). They have been used to study for instance the emergence and maintenance of ISAV in Europe (Nylund et al., 2003) and the distribution of different genotypes of VHSV (http://www.fishpathogens.eu/vhsv/geo_distribution.php). Generally, the marine isolates of VHSV identified to date produce low or no mortality in immersion infection trials on rainbow trout and Atlantic salmon (Skall et al. 2004, Snow et al. 2005). However, virus from the VHS outbreaks in sea farmed rainbow trout at the west coast of Sweden during 1998-2000 and in Finland 2000-2004 cluster to the Genotypes 1b and 1d, respectively (Einer-Jensen et al., 2005; Raja-Halli et al., 2006). For the Finnish outbreaks, a marine source of infection has been suggested. The virulent and non-virulent strains of VHSV are genetically closely related (Betts & Stone 2000, Einer-Jensen et al. 2004, Snow et al. 2004). Sequencing analysis of the entire coding regions of the genome indicates that only a small number of amino acid residues may be involved in the determination of VHSV virulence in rainbow trout (Betts and Stone, 2000). Furthermore, molecular epidemiological studies of nodavirus indicate that a cluster with barfin flounder nervous necrosis virus (BFFNNV) is associated with cold-water species but a cluster specific to cod has been suggested (Nylund et al., 2008).

Pulsed field gel electrophoresis (PFGE) is a method that can discriminate between highly related strains of bacteria and is commonly used in epidemiological studies of pathogenic organisms. In Japan for instance, strains of *F. psychrophilum* were classified by this method into 20 clusters and 42 genotypes. Results suggest that *F. psychrophilum* isolated from different fish are genetically different and strains with several PFGE types have spread within Japan (Arai et al., 2007). PFGE characterisation of *V. anguillarum* serotype O1 showed that isolates from Scandinavia and southern Europe belonged to two different clonal lineages, but the strains did not have any host adaptation (Skov et al., 1995).

In phylogenetic and epidemiological analyses of closely related bacteria the Multi-Locus Sequence Typing/Analysis (MLST/A) method has been used. It is based on PCR amplification of several housekeeping genes that are spread around the bacterial chromosome. These housekeeping genes are essential for the survival of the bacteria and are therefore little changed during development. Difference in gene sequence are used to define allelic profiles or sequence types and the relatedness among isolates is determined by comparing the sequences (Maiden, 2006). The MLST schedules developed (Maiden, 2006) have been used successfully to explore the population structure of bacteria, to study the evolution of their virulence properties and to identify antibiotic resistant strains and epidemic clones (http://mlstoslo.uio.no/index.html). However, this method does not discriminate between a very homogenous group of fish pathogenic bacteria such as *A. salmonicida* subsp. *salmonicida* or between different subspecies of *A. salmonicida* (Colquhoun, 2007). Highly related isolates based on MLST may have minor changes in the chromosome that changes the PFGE and multi-locus variable repeat (MLV) profile dramatically (Wilson et al., 2009). For instance, it has been shown that the MLST does not have the necessary discriminatory power to study epidemic spread of *Staphylococcus aurus* (Melles et al., 2007).

Multi-locus variable repeat analysis (MLVA) is a more sensitive method with high discriminatory power. Sequencing of bacterial genomes has revealed that they also contain a high number of multiple loci with small repetitive nucleotide sequences. These repetitive short DNA sequences may be a result of DNA polymerase slippage and recombination during chromosomal DNA replication (Tuntiwechapikul and Salazar, 2002). If these events happen with high frequency it will result in variations in the number of each of the tandem repetitive sequences (VTNR). Using PCR to amplify these stretches of DNA, the sequences surrounding the VTNR has to be known. Specific primers have to be generated and the PCR products will be of varying fragments length if the DNAs compared have variable numbers of repeats. These differences in size result in various allele profiles and have high discriminatory power. They have been used to trace bacteriological contamination, follow the spread of pathogens in the environment, study disease outbreaks and for forensic purposes (van Belkum, 2007). Pathogenic bacteria are often very homogeneous, but genome sequencing has revealed genomic differences among homogeneous groups like the human pathogens *Yersinia pestis* (Kingston et al., 2009) and *Vibrio cholerae* (Olsen et al., 2009) and the fish pathogens *A. salmonicida* (Colquhoun, 2007; Tandstad et al., 2009) and *V. anguillarum* (Tandstad et al., 2009). To establish the MLVA method for a specific organism the bacterial genome sequence has to be known and VTNRs chosen to be included in the MLVA has to be optimal. Strains must be passaged several times and re-typed in order to check that the VTNR loci chosen are stable and reproducible. This is especially relevant to VTNRs that are present in coding regions or promoters, or in species that use contingency loci (Lindstedt, 2005). PCR inaccuracy, because Taq polymerase may be unreliable in copying the repeats, leads to artificial changes in the repeat profile (van Belkum, 2007).

Population analysis methods such as Denaturating Gradient Gel Electrophoresis (DGGE) are capable of analysing 16S rDNA without cultivating the bacteria, hence giving a picture of the total diversity of bacterial DNA present in a sample. Despite being a relatively old method, it

has been applied in aquaculture to describe the bacterial communities associated with early life stages of cod (Brunvold et al., 2007; McIntosh et al., 2008), halibut (Jensen et al., 2004), haddock (*Melanogrammus aeglefinus*) (Griffiths et al., 2001), rotifers (Rombaut et al., 2001) and scallops (Sandaa et al., 2003). The method is particularly useful as an initial investigation for distinguishing between communities and identifying the numerically dominant community members. For instance, comparisons between groups with either high or low mortality will be facilitated, as the DGGE protocols enables rapid overview of the presence or absence of certain bands. DNA from the bands will be sequenced and used for identification of the important strains. Important bands can also be used to design probes/primers which in turn may be used in PCR protocols for the detection of certain pathogens in the different compartments of aquaculture systems (Brunvold et al., 2007; Sandaa et al., 2008) and proliferation of opportunistic pathogens (Sandaa et al., 2008). DGGE can rapidly screen multiple samples and obtain valuable information about community changes and differences, giving an indication of the sources of contamination and sites of proliferation of different strains.

Microsatelite DNA loci are highly variable and considered selectively neutral (not adaptive). Thus, they offer a powerful and commonly used means of assessing and comparing the genetic structure of populations. Analysis of microsatellite DNA variation for instance showed no significant differentiation of *L. salmonis* populations sampled from wild and farmed salmonids throughout the North Atlantic (Todd et al., 2004).

Mitochondria have high mutation rate at the DNA level and they are maternally inherited and therefore very suitable for genetic studies (Shearer et al., 2002). *L. salmonis* phylogeny has been studied by sequence variation of four mitochondrial genes (Tjensvoll et al., 2006). *L. salmonis* from Norway, Scotland and Russia had low variation, and only a weak degree of sub-division was found between lice from Canada and the North-East Atlantic. In contrast, all

Atlantic samples were highly different from a Japanese sample. It was suggested that the lack of genetic differentiation between North Atlantic samples was mediated by passive transport of larvae as well as salmon migratory patterns.

3.3 Methods to study transfer of diseases between species

When studying risk of disease transmission and susceptibility, injection challenges have been common (Devold et al., 2000; Korsnes et al., 2005). It could be argued that challenge by injection followed by a negative result would be a sound method to rule out the potential for disease transfer, but a positive result would be harder to defend. The method is highly artificial and does not mimic disease transfer in a natural situation. In contrast, cohabitant models mimic the natural situation to a higher degree. At Nofima Marin in Tromsø, Norway, a novel challenge model to study transfer of disease between fish species has been developed (Mikkelsen et al., 2009). It is composed of a tank system designed to mimic natural transmission of waterborne diseases in order to assess infection risk and spreading of disease between locations. The effluent from the tank with infected fish is mixed with clean water in the receiving tanks. This tank system has a great advantage over cohabitant models as transmission of disease through physical contact between healthy and infected or moribund fish is excluded. *V. anguillarum* serotype O2a, known to cause vibriosis both in salmon and cod (Larsen et al., 1994), was transferred through effluent from infected salmon to naïve cod and resulted in 60 % mortality in cod. Salmon was shown to be susceptible to i.p. challenge with *V. anguillarum* serotype O2b, but the dose chosen resulted in considerably lower mortality in salmon compared to cod (36 and 86 %, respectively). Hence, the risk of vibriosis outbreaks in salmon due to serotype O2b appears to be very low. Transmission via effluent of atypical furunculosis from infected halibut to cod was also demonstrated (Mikkelsen et al., 2009). Attempts to transfer typical *A. salmonicida* subsp. *salmonicida* to different marine fish species, including i.p. challenge and bath challenge, demonstrated that this disease does not

constitute a major threat to turbot, cod, halibut and wrasse (Bergh et al., 1997; Hjeltnes et al., 1995).

A recently published paper illustrates how challenge methods greatly affect the results in susceptibility studies (Urquhart et al., 2009). Both i.p. injection, cohabitation and immersion routes of infection were used to determine the susceptibility of cod juveniles to IPNV isolated from salmon. Mortalities after immersion challenge (17 %) were not significantly different from uninfected controls. Also, mortality rates were low when challenged by cohabitation (20 %) compared to i.p. injection (100 %), suggesting that cod juveniles have low susceptibility to IPNV when challenged by natural routes. Snow et al. (2009) studied the susceptibility of Atlantic cod juveniles to a genotype III VHSV isolate from wild caught cod by using oral, immersion and i.p. infection routes. High mortalities and clinical disease occurred in i.p infected fish but no evidence of infection was obtained following immersion or oral challenge, despite the use of a highly sensitive Real Time PCR detection method. Thus, there was a natural resistance to infection in cod following simulated natural conditions.

3.4 Survey methodology and studies of diseases in wild fish populations

The development of survey technology has not been the same as for the molecular tools used for pathogen detection. The survey programmes for notifiable diseases are carried out by methods subject to quality control by the National Food Safety Authority, ensuring a certain number of sampling sites and individuals per sampling. On the other hand, surveys of wild populations are generally characterised by few resources and only incidental sampling. Examples of advanced survey technologies being applied to other research areas are given by Bogstad et al. (1995), describing cost-efficient survey designs to assess food consumption by different wild fish species, taking into account biases in distribution of starved fish versus fish that has had access to food. The statistical problems are similar to those caused by pathogen distribution that could lead to an underestimation of the prevalence of pathogens. It could

therefore be recommended that more advanced statistical methods may be applied when designing surveys for prevalence of pathogens.

Very few larger survey programmes have been carried out in Norwegian waters. VHSV has for instance been subject to systematic sampling in north European waters (reviewed by Skall et al. 2005). Unfortunately sampling from fish in Norwegian waters was restricted to the open sea areas (Brudeseth and Evensen, 2002) and not to coastal fish. However, the coastal zone and fjords may be critical for transmission of disease from wild to cultured fish. During certain periods of the year large numbers of pelagic and mesopelagic fish, for instance herring, migrate into the fjords, particularly during spawning.

An attempt to systematically survey wild fish in the Norwegian coastal waters for *F. noatunensis*, has been published by Ottem et al. (2008) using Real Time PCR as the detection method. However, the total number of samples and sampling sites were too low for anything but a general conclusion that a geographical bias in the distribution of the pathogen exists.

Heuch et al., (2007) monitored *C. elongatus* infections of wild coastal fish on the southeast coast of Norway at various times during 2002 to 2004. Several different sampling techniques were used to obtain a large variety of fish species and sizes in different habitats. The lice prevalence for all coastal fish (n = 4427) pooled was 15 %, but there were great differences between fish species and season (Heuch et al., 2007). North Sea lumpfish, *Cyclopterus lumpus* L., spawners were the most infected fish followed by gadoids. Sea trout and herring also carried *C. elongatus* at moderate prevalence values. The relatively high numbers of chalimii on lumpfish suggest that offshore fish sustain an oceanic population of this louse species.

An extensive IPNV survey has been performed recently in Scottish waters with sampling from 30,000 marine fish comprising 37 species in the vicinity of aquaculture farms during a 16 months period (Wallace et al., 2008). The survey technology and pathogen detection methodology used is reliable and the study has brought new knowledge about marine IPNV reservoirs and pathogen exchange between wild and farmed fish populations. Demersal trawling, as well as rod and line were used to catch fish. Tissue culture methodology (Munro et al., 2004) combined with confirmation by ELISA (Smail et al., 2003) were used for pathogen detection. In total 45 isolates were obtained from nine different species, but only two samples were above detection levels for the titration method. Future studies should examine the virulence of these two isolates to farmed Atlantic salmon. The estimated prevalence of IPNV in the Scottish marine environment was low at 0.15 %, but in fish caught less than 5 km from IPN-positive fish farms the prevalence was significantly higher (0.58 %). This prevalence persisted and did not significantly decrease during the 16-month study period. The estimated prevalence of IPNV for each positive species was less than 1 % with the statistically non-significant exceptions of flounder, *Platichthys flesus* (L.) and saithe. Overall, the survey provided evidence that clinical outbreaks of IPN in farmed Atlantic salmon may cause a localized small increase in the prevalence of IPNV in wild marine fish illustrating these as reservoirs of IPNV in Scottish waters. The role of these reservoirs in the re-infection of farmed salmonids is not understood. Wild fish reservoirs may not currently be a major factor in the infection of fish farms, although they could be important in the re-emergence of infection if the prevalence of IPN in the salmon farming industry were to fall to very low levels.

In contrast to the survey in Scotland, a high prevalence of viral infection was reported in wild salmon in northern Spain (Bandin and Dopazo, 2006), where a non-lethal method (Cutrin et al., 2005; Lopez-Vazquez et al., 2006) was used for detection of different viruses in fish blood leukocytes. Real Time PCR/nested PCR was used for analysis in a two-year Atlantic salmon

restocking campaign, to ensure that brood stock used for fry production and stocks of fry employed for restocking were free of the viruses IPNV, IHNV and VHSV. The percentage of fish carrying any of these viruses varied between 47- 50 % and only a minor part of the captured breeders could be used in the restocking programme. IPNV prevalence was up to 39 %. In certain cases co-infection of two or even all three viruses was observed. Considering that the migration pathways of the salmon returning to Galicia include feeding areas that are shared with wild Atlantic salmon populations of other areas of Europe and North America, such high levels of virus carriers is worrying. However, the Real Time PCR method used in this study did not detect infectious, replicating viruses and thus the impacts of these findings are uncertain.

A comprehensive report on sea lice and the interaction between wild and farmed salmonids has been published (Revie et al., 2009) also describing the challenges in designing good survey methodology. Sampling method and bias are important issues when studying the abundance and distribution of parasites in wild fish. Often there are limitations in sampling procedures. For instance capture of fish in fresh and brackish water or capture using methods that could be lethal to the fish may lead to underestimation of sea lice numbers. Under these circumstances sea lice tend to detach from the host quickly. To avoid killing by the netting process, nets can be placed in the sea for very short periods of time. However, this is very labour intensive and restricts the number of nets or locations to be fished in an area. When catching by hooks, fish may also die prior to retrieval. New field survey technology has been developed in recent years. For instance, catching and retaining live smolts in sealed boxes is found to give minimal scale loss and thus more reliable estimates of sea lice levels (Holst and McDonald, 2000). Sentinel cages containing naïve smolts have also been used successfully to estimate local infestation pressure on wild salmonids (Bjørn et al., 2008; Bjørn et al., 2009; Boxaspen and Asplin, 2008; Finstad et al., 2007).

Data sets over longer time periods make it possible to assess infection across years. Time-series data from more than seven years have been collected for *L. salmonis* and *C. elongatus* abundances on wild salmon captured in coastal bag nets in marine waters off the north coast of Scotland (Todd et al., 2006). The fish remained live and free- swimming in these traps and thus the data were more reliable. Also, time-series modelling can increase the understanding of parasite dynamics. McKenzie et al. (2004) described the pattern of infection for *C. elongatus* on Scottish fish farms focusing on patterns within years (or production cycles).

Mathematical modelling combined with field data could be a powerful and more reliable tool to study transmission dynamics (Krkošek et al., 2005; Krkošek et al., 2006b). However, precaution should be taken because modelling relies on detailed empirical data which is often lacking from wild fish. Confidence in the observational data is fundamental to any model. Sampling is not easy and often one lacks the opportunity for microscopic inspection in the field. It is then difficult to discriminate between stages and species. To get reliable data, one should bring together sea lice data and hydrographic models into an integrated framework. Furthermore, there is a need for more sophisticated multiple variable analyses.

4. Evaluation of the risk involved in pathogen exchange between farmed and wild fish

Introduction

The risk involved in any operation is related to the likelihood of undesirable events occurring, known as hazards. The risk of a hazard occurring combines the likelihood and the consequences of occurrence. The hazard which is addressed in the present paper is the emergence of disease in farmed or wild fish populations.

Risks of acquiring and spreading diseases are always present in food production, regardless of the foods that are produced or systems that are used for production. However, fish-farming has some unique characteristics worth considering, as well as some characteristics that are common to farming of livestock. Unique to fish farming is, naturally, that it is conducted in

water and often in open net-pens that have no barriers to pathogen exchange with the environment. Water flows freely through the cages and potential pathogens may come in contact both with wild fish and other farmed fish populations. These open systems are also vulnerable to escape of farmed fish. The likelihood of pathogen exchange between wild and farmed fish is enhanced by the attraction of persistent shoals of wild fish to fish farming cages due to feed being available or due to shelter (Dempster et al., 2009). Furthermore, the fish farming industry is commonly divided into juvenile and grow-out production. Producers of juvenile fish typically serve many grow-out farm sites. Hence fish, and potentially pathogens, are moved over large distances (Lyngstad et al., 2008).

In common with other livestock production, fish are farmed in large, dense populations. The scale of production of farmed fish may vastly exceed that of natural production of the same species. This may have implications for the quantities of pathogens produced, and hence the infection pressure to which both farmed and wild fish are exposed (Krkôsek, 2010). Dense and large farmed populations may also promote selection towards increased virulence in pathogens (Pulkkinen et al., 2010).

4.1 Marine aquaculture production in Norway

Open net-pen systems are the predominant production units used in marine fish farming in Norway and legal concessions are administered by the Directorate of Fisheries (DF; www.fiskeridir.no). All licenced sites, and the concessions these are attached to, are registered in open access domain. A total of 922 marine sites farmed Atlantic salmon or rainbow trout (salmonids), 285 farmed other marine fish species and 283 farmed shellfish at some point in time during 2008 along the Norwegian coast. The production of salmonids is by far the largest enterprise in Norwegian aquaculture. Operators of marine sites holding salmonids are required to report key production statistics to responsible authorities on a monthly basis (Kristoffersen et al., 2009). A summary of some of the statistics (Figure 1) show a declining trend in the number of marine sites holding salmonids over the years 2003 – 2009, whereas

the total stock and biomass of farmed salmonid fish show an increasing trend. In 2008 the total stock varied between 250 – 350 million fish, and the biomass varied between 4 – 6 hundred thousand tonnes (Fig. 1).

4.2 Open production units

Net pens allow pathogen exchange with the surrounding environment. This is a recognised and widely debated problem for sea lice in salmon farming, where planktonic larval stages produced on fish in the pens drift passively into the surroundings with the water current. The spread of other pathogens by passive drift in the water current has also been suggested in a number of studies. Most of these studies rely on the tendency for disease outbreaks to cluster in space and time. This is well documented for ISA in Norway (Jarp and Karlsen, 1997; Scheel et al., 2007; Vågsholm et al., 1994), Canada and USA (McClure et al., 2005) and Chile (Mardones et al., 2009) and for PD in Norway (Kristoffersen et al., 2009). Phylogenetic studies confirm that ISA-virus isolates from clusters of outbreaks are related (Lyngstad et al., 2008). These studies have all taken into account horizontal transmission of ISAV and SAV, and the possibility of passive drift of the causal agents. However, in order to disentangle transmission by passive drift from other transmission pathways that are dependent on distance, it is necessary to determine the role that water currents play. Gustafson et al. (2007) showed that tidal currents affected the spread of ISA in the Quoddy region of Atlantic USA and Canada. Viljugrein et al. (2009) concluded that the spread of PD in an area on the west coast of Norway was significantly better predicted by using hydrodynamic modelling to estimate water contact between farm sites, than by simply using distance between farm sites. Hence, these latter two studies address effects of passive drift of pathogens directly, which clearly emphasizes the potential for pathogen exchange between fish farms and the local environment.

A further characteristic of net-pen production units is that they are vulnerable to escape of fish. Escapes of fish must be reported to the DF which summarises yearly figures of officially reported escaped fish (Fig. 2). These figures show that in some years more than 1 million farmed fish have escaped and in some instances escaped fish have also been diagnosed with disease. Recently, there were two instances of reported escapes of Atlantic salmon from two different sites with ISA in Troms County, North Norway. Escaped salmon were later caught in two different local rivers, and ISAV was isolated from these fish (Johansen et al., 2009b). These instances clearly demonstrate the potential for pathogen exchange from escaped farmed fish to wild fish. However, the Norwegian fish farming industry have increased efforts to reduce escapement. Especially for Atlantic salmon, this seems to have been effective since numbers of escapees have declined markedly in recent years (Fig. 2).

4.3 Pathogen transmission by wild fish

Open net-pen production systems are known to attract assemblages of wild fish with a high diversity of species both in warm temperate marine waters (Dempster, 2004; Tuya et al., 2006) and in cold marine waters (Dempster et al., 2009). In Norwegian salmon farming, farm-aggregated biomasses of the four dominant species have been estimated to be an average biomass of 10.2 t farm^{-1} (Dempster et al., 2009). These assemblages of wild fish imply that farmed and wild fish are in close contact and wild fish may act as disease vectors of pathogens and parasites among salmon farms. Saithe, which is the most abundant fish species associated with Norwegian salmon farms (Dempster et al., 2009), has been suggested to act as a natural reservoir of SAV (Graham et al., 2006) and is a carrier of IPNV (Wallace et al., 2008). Movements of wild fish between adjacent farms may contribute to the spread of pathogens and propagation of disease outbreaks. Uglem et al. (2009) studied the spatio-temporal distribution of wild fish in a fjord system with intensive salmon aquaculture in Norway. Saithe equipped with acoustic tags typically had periods of residence at specific farms interspersed with rapid and frequent movements to adjacent farms. The authors

concluded that salmon farms should be considered to be connected through wild fish movements with regard to potential pathogen transmission (Uglem et al., 2009). Even though direct evidence for pathogen transmission through wild fish is lacking, the observed aggregations of wild fish associated with fish farms and frequent movements between farms, clearly addresses a potential for disease spread between fish farms through wild fish.

4.4 Large dense farm populations

Hansen et al. (2006) estimated the population of homing Atlantic salmon to Norwegian rivers to vary between 0.5 – 1 million individuals over the years 1995 – 2005. These figures are comparable to the annual number of escaping salmon (Fig. 2). However, given that the figures of Hansen et al. (2006) are representative also after 2005, the standing stock of marine farmed Atlantic salmon on the Norwegian coast outnumber the homing wild salmon population by factors of 250 – 700 (Fig. 1). This vastly increased population of farmed salmonids that are constantly present in the coastal waters certainly has the potential to accumulate pathogens that are shed by infected hosts, e.g. sealice (Heuch and Mo, 2001). Murray (2009) explored deterministic mathematical models where different forms of pathogen transmission were formulated. Model III depicted pathogen transmission through the water where infective stages can persist away from the host. Infection may then result from exposure to pathogen-contaminated water, rather than close contact with infected hosts. Pathogens accumulate in the water as they are shed by infected hosts and the more shedding hosts there are the higher the local concentration of pathogens is likely to be, for given pathogen decay and dispersal rates. In turn, increasing pathogen concentrations will increase the rate at which susceptible hosts become infected. Hence, the rate at which susceptible hosts become infected increases non-linearly with the number of infected hosts (Murray, 2009). Density dependent shedding rates and transmission processes may well explain the tendency for large epidemics to emerge in aquaculture, such as recent outbreaks of ISA in Chile (Mardones et al., 2009) or PD in Norway (Kristoffersen et al., 2009).

4.5 Evolution of virulence

Ecological changes may trigger the emergence of infectious diseases and affect pathogen virulence through natural selection. Pathogen virulence is often related to the pathogen´s ability to propagate and transmit to susceptible hosts (Anderson and May, 1991). In fish farms the density of susceptible hosts is unnaturally high. Once a pathogen is introduced to a farm, the probability of an encounter between an infected and a susceptible host is increased compared to natural systems. The probability of transmission per contact may also increase since farmed populations consists of homogenous groups of fish (Ebert, 1998), or since the fish may be stressed by crowding or confinement to stressing environmental conditions (Sniezko, 1974). In the theory of evolution of virulence, high densities of susceptible hosts will promote increased virulence since rapid propagation of a pathogen within an infected host will affect infectivity of this host and lead to higher rates of transmission in dense host populations. However, such rapid pathogen propagation within infected hosts will also theoretically increase host mortality rate and hence decrease the host infectious period. Therefore, in less dense host populations, pathogen virulence will decrease since the number of secondary infections arising from the introduction of an infected host into a susceptible population (R_0) will depend more on host infectious period (Anderson and May, 1991). On an ecological timescale, such selective forces have been suggested to shape the co-evolution of the myxoma virus and wild populations of rabbits in Australia (Fenner, 1983). Artificially high population densities have also been suggested to be the underlying cause of recurrent emergences of new infectious diseases in aquaculture (Murray and Peeler, 2005). Circumstantial evidence suggests increased virulence of pathogens causing VHS (Einer-Jensen et al., 2004) and ISA (Nylund et al., 2003) in fish farms compared to wild fish. Whether this is due to pathogen evolution or the artificial farm conditions, however, is not clear. Long term studies from Finland on the evolution of *Flavobacterium columnare*, causing columnaris disease, suggest that this bacterium has evolved towards increased virulence

(Pulkkinen et al., 2010). It is argued that aquaculture conditions have enabled virulent strains of *F. columnare* to spread and cause outbreaks. Among many correlating arguments to support this, it is shown that dead fish stay infectious and greatly enhances transmission of the pathogen. Hence, virulent strains of the pathogen are less compromised by killing their host in terms of transmission (Pulkkinen et al., 2010).

4.6 Large scale movements

Fish and fish products are constantly moved around the globe due to trade or with the aim to spread fish to new areas. Live salmonids have through all times been distributed by man to new areas, both locally and globally. More recently, with the growth of salmonid aquaculture, salmonids are traded extensively both as embryos and live fish, and both internationally and locally. This extensive movement of fish implies a risk of movement of pathogens. Although movement of diseased fish probably is non-intentional, low disease prevalence or sub-clinical infections might go undetected. An example from international trade involves the recent emergence of ISA in Chile. Phylogenetic analyses of ISAV from Chile suggest that the virus originates from Norway (Vike et al., 2009) and that it may have been introduced some time around 1996 (Kibenge et al., 2009). There has been extensive export of salmon eggs from Norway to Chile, and infected eggs have been speculated to be an introductory pathway (Vike et al., 2009). Within Norwegian salmon farming, there is extensive shipment of live fish along the coast. For example, there is generally a larger demand for smolts in the north than what are produced locally. Hence fish are shipped over large distances from the south to grow out farms in the north (Lyngstad et al., 2008). This activity has been speculated to be the cause of isolated outbreaks of PD in the north (Kristoffersen et al., 2009).

A large risk associated with trade and movement of fish and shellfish involves imports of exotic pathogens. Characteristic for many incidents of imported exotic pathogens is that they have had large consequences for wild native populations and that such consequences were

unpredictable since pathogenicity evolved as new native species were infected. Examples include mass mortality of European oysters and European crayfish caused by pathogens imported from American species (Alderman, 1996; Comps, 1978) and the introduction of the nematode *Aguillicola crassus* to the European eel through introductions of Asian eels (Koops and Hartmann, 1989). A prime example from Norway, which depicts an intricate interaction between fish farming and wild fish, is the monogenean parasite *G. salaris*. It is well documented that this parasite was initially introduced to a fish farm in Norway during the early 1970s and spread to Norwegian salmon rivers through cultivation practices (Johnsen and Jensen, 1986; Johnsen and Jensen, 1991). Later this parasite has probably spread further by migration of infected fish through brackish waters in the fjords (Jansen et al., 2007; Johnsen and Jensen, 1991). However, both historical records (Johnsen and Jensen, 1991), morphological studies (Mo, 1991) and phylogentic studies (Hansen et al., 2003) suggest multiple introductions of *G. salaris* to Norway, and that these incidents are associated with rainbow trout and Atlantic salmon farming. *G. salaris* now has a history of infection in 46 Norwegian salmon rivers (Jansen et al., 2007). The history and effects of this epidemic are summarised in Bakke et al. (2007). A study of the genotypes and sub-groups of IPNV present in farmed and wild salmonid fish in Ireland highlight the importance of import risk analysis for diseases not listed under current legislation (Ruane et al., 2009). All isolates found belonged to genogroup 5 and they could be divided into two subgroups. All reported clinical outbreaks of IPN were associated with subgroup 2 which consisted of isolates from imported farmed stock. Isolates from wild fish were identical to some isolates from subgroup 2, and therefore are believed to have originated from infected farms. There are other examples of exotic pathogens having detrimental effects on local fish and shellfish populations (Murray and Peeler, 2005; Peeler and Thrush, 2009). It may be argued that the long history of serious and unforeseen consequences of imports of exotic pathogens puts large scale movements of live fish on the top of the list of risky practices.

5. Future research

5.1 Pathogen levels – wild fish surveys

Limited research has been undertaken on diseases of wild fish at the population level and on the interactions between wild and farmed populations in terms of exchange of pathogens. To be able to decide to which extent farmed fish contribute to spreading of pathogens and to decide which effect this has on the wild populations, surveys to map pathogen distribution in wild populations are necessary. These surveys should extend over a long time period and need to be performed both in areas with fish farms and distant from fish farms. To our knowledge no such large scale wild fish surveys have been performed in Norwegian waters. Material from one survey could be analysed for the presence of several pathogens. However, which organs and detection methods that should be used must be taken into careful consideration. If the sampled materials from surveys are stored it would be possible to perform retrospective studies when new pathogens emerge in fish farming.

Specific disease outbreaks should be followed up by pathogen screening of wild fish surrounding the affected farm. Wild salmonids from nearby rivers ought to be included in such studies. For parasites with indirect lifecycles, areas around fish farms should also be monitored for the presence of intermediate hosts.

To our knowledge no study has addressed changes in pathogen prevalence and intensity in wild fish before and after establishment of intensive aquaculture, and in the case of salmonid farming in Norway, this would be too late. However, Atlantic cod aquaculture is now being established in fjords without prior aquaculture activities, which gives a unique opportunity to study changes in parasite, bacterial and viral distribution in wild coastal cod. Similar data can be gathered from fjords with and without aquaculture and in closely-aquaculture-associated wild marine fish (Dempster et al., 2009). Such data could be used to develop models for carrying capacities and management measures in intensively farmed Norwegian fjord

systems.

5.2 Increased basic research on pathogen virulence

Gaining knowledge about virulence factors is important in terms of pathogen exchange between wild and farmed populations. In wild fish surveys genotyping of virulence determinants ought to be included to resolve whether high or low virulence variants of pathogens are present and whether the prevalence of these variants changes over time. This will give some indications about the likelihood of the pathogen to cause disease in surrounding fish populations and may have implications for disease management. In addition, there is a need to obtain more information on the taxonomic subgroups of highly diverse groups of pathogens.

5.3 Developing challenge and transmission models for selected pathogens

Challenge models are used to study susceptibility and virulence, routes of pathogen transmission within a species and between different species, pathogen shedding rates and infectious dose required to induce infection. Pathogens isolated from wild fish should be characterized and tested in experimental challenge studies to examine whether they are infectious to other species. Transmission studies could also decide whether wild fish, that are not susceptible to a pathogen itself, can act as vectors and spread pathogens in and between farms. Experimental shedding and infection experiments in a controlled environment in combination with modelling of pathogen spread in fjord systems will also increase the knowledge about risk of shedding and spreading of pathogens from wild fish populations.

5.4 Understanding the consequences of and minimising the occurrence of sub-clinically infected carriers

Fish pathogens are able to establish persistent infections that are characterized as infections where the micro-organism is not cleared, but remains in specific cells of infected individuals

without rapidly killing or even producing damage of the host cells. For IPNV for instance it is known that survivors of disease outbreaks become persistently infected and establish a life long carrier condition. Persistently infected fish are present both among farmed and wild fish populations and in various fish species. However, the impact of persistent infections on pathogen exchange between farmed and wild fish and *vice versa* is largely unknown. There is limited information regarding the amount of virus shed from persistently infected fish and amounts of virus required to induce infection through the water. Recent studies provide some information regarding these aspects in experimental IPNV and ISAV infections (Gregory et al., 2009; Urquhart et al., 2008). Environmental and host stress may cause recurrence of disease in virus carriers (Johansen et al., 2009a; Taksdal et al., 1998). It is central to gain more information about which stressors are most likely to induce recurrence in farmed fish and accordingly avoid these stressors in fish husbandry practice.

5.5 Breeding for disease resistance and developing more efficacious vaccines

Disease resistance as a breeding goal has been implemented by the breeding companies for some years and increased resistance to IPNV in Atlantic salmon due to breeding has been shown (Kjoglum et al., 2008). Together with vaccination this is an important approach to prevent disease in farmed populations and thereby also prevent transmission of pathogens to wild fish populations. Development of efficient fish vaccines has had a major positive effect in providing protection against disease outbreaks (Sommerset et al., 2005) and in some cases to prevent or reduce the spread of pathogens from farmed to wild fish. However, not all vaccines are sufficiently effective and for many pathogens vaccines do not exist. It has for instance proven difficult to develop effective vaccines against viruses and intracellular bacteria. Increased knowledge about pathogen-host interactions and cell-mediated immune responses leading to development of new vaccine concepts will be necessary to prevent disease and transmission of these pathogens. However, it is also unknown whether vaccinated

or disease resistant fish are able to clear viral pathogens when infected or if a carrier condition is established.

5.6 Investigating possible reservoirs and vectors

Better understanding of the role of reservoirs and vectors in virus transmission is needed, in particular that of wild piscivorous birds and invertebrates. Based on recent findings (Petterson et al., 2009b), it would be interesting to study the possibility of pathogen transmission by salmon lice.

5.7 Modelling disease spread in salmon farming

Due to the extensive size of the population of farmed salmonids compared to that of the wild salmonids in Norwegian marine waters, it can be argued that knowledge on disease dynamics and disease spread in salmon farming is also a key for generating expectations for the wild population. The documentation of Norwegian salmon farming production in the marine environment is unique within worldwide aquaculture. Farmed salmon are produced on farm sites that are geo-referenced in a central aquaculture register. Furthermore, vital statistics on farmed salmon stocks can be documented many years back in time on a monthly basis. This is due to a system of mandatory monthly reporting of key production statistics (see Kristoffersen et al., 2009). These data, in combination with historical data on disease incidences or sealice abundance in farmed salmon stocks, yield unique possibilities for space-time modelling of disease spread in salmon farming. Recent progresses in the development of large scale stochastic models for the spread of viral diseases in salmon farming were founded on combining farmed fish stock data with disease incidence data (Aldrin et al., 2010; Kristoffersen et al., 2009; Scheel et al., 2007). In parallel, increasing efforts are put into hydrodynamic modelling along the Norwegian coast. Hydrodynamic models used to estimate reciprocal relative water contact between local salmon farm sites, have also been integrated with statistical models on disease spread (Viljugrein et al., 2009). The integration of

hydrodynamic models and disease spread models may contribute to quantify the risk of disease spread through passive drift of pathogens in the water current, as well as estimating the degree of potential infectious contact between sites. When disease spread models reasonably portray historic disease incidents, such models can be used as objective tools to simulate effects of intervention strategies. For example, what is the effect of depopulation of infected farms, vaccination of fish with given protections or relocating farms sites? The development of such tools is facilitated by continually increasing computational power. However, development of simulation models will demand high interdisciplinary collaborating efforts between expertise in fish diseases, mathematical modelling and statistics, and hydrology. Useful models will also rely on accessibility of high resolution data covering key variables of importance to disease propagation and spread.

5.8 Parasites – transmission and identification

Marine parasites range from relatively simple organisms with direct transmission between hosts to animals with complex lifecycles. Marine parasitology is therefore a diverse and challenging field of research. However, experience from salmon farming clearly shows that one of the greatest environmental problems is connected to transfer of parasites between wild marine fish and mariculture. The transfer of parasites between farmed species as well as between wild and farmed species is therefore an important area of future research as mariculture grows and diversifies. Correct identification of the organisms is essential in parasitology (see Section 3.1) and molecular markers can now be used to identify parasitic organisms with complex or even cryptic morphology (Hansen et al., 2007). Commercial companies as well as research institutes now offer Real Time PCR analysis for detection of e.g. a microsporidium and a myxosporidium and similar techniques should be developed for other parasites. In addition, more easily accessible alpha taxonomy protocols should be developed.

5.9 Future research - Concluding remarks

Multiple factors interact and influence the outcome of pathogen transmission and disease development. With the possible exception of salmon lice, there are few reliable data sets on the distribution of fish pathogens in wild populations, and the knowledge of interactions with wild reservoirs is thus limited. Furthermore, the susceptibility in host-parasite relationships is in many cases limited or unknown. Modelling disease spreading without knowing the transmission processes may be speculative or at least of limited value. It can be hypothesized however, that increased population density enhances disease proliferation unless improved prophylactic measures are applied as an integrated part of the development of the aquaculture industry.

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Figure 1. Summary statistics for Norwegian production of marine farmed Atlantic salmon and Rainbow trout from 2003 to 2009. The upper panel shows the number of marine sites holding salmonids, the mid panel shows the total stock of farmed salmonids (in millions) and lower panel shows the total biomass (in hundred thousand tonnes), at any point in time.

Figure 2. The number of officially reported escapees of Atlantic salmon, Rainbow trout and Atlantic cod over the years 2001 – 2009 (source: Directorate of Fisheries).

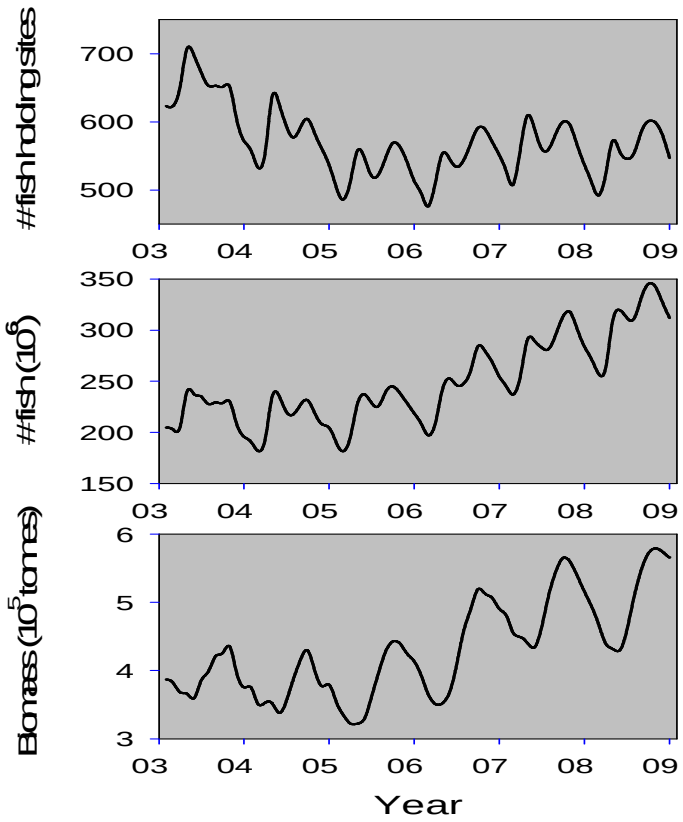

Figure 1

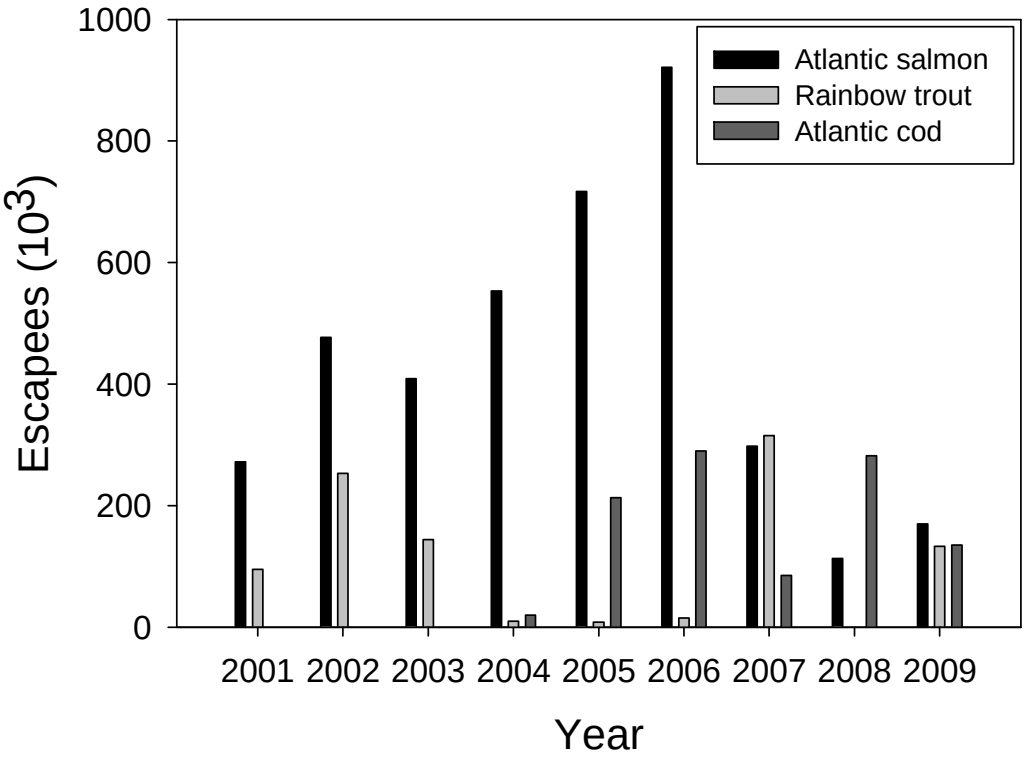

Figure 2